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Influences of microbial community structures and diversity changes by nutrients injection in Shengli oilfield, China

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ABSTRACT

After dozens of years of crude oil recovery, oilfields are facing increasing depletion of oil resources. More than 60% of the Earth's oil resources are likely still in the subsurface oil deposits. Although related theories and technologies of enhanced oil recovery are not routinely used, microbial enhanced oil recovery (MEOR) has the potential to be one of the most valuable technologies in crude oil exploitation. The diverse microbial communities found in oil/water samples from the production well of the Shengli oilfield were analyzed by culture-independent molecular techniques, including a 16S rRNA gene clone library, terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE). In this study, nutrients (carbon source, nitrogen source, inorganic salts and trace elements) were injected into S12-4 wells, and the microbial diversity, composition, dynamics, quantity and structures were subsequently determined from samples extracted 0–17 months after the nutrient injection. The results show that the nutrient injection had a profound effect on the diversity, composition, and relative number of the petroleum microorganisms. The dominant species in the S12-4 well were mainly of the genus *Pseudomonas*, belonging to the order Pseudomonadales and *Marinobacter*, of the order Alteromonadales, as well as a number of methanogenic archaea.

This study is the first time microbial communities in an oil reservoir have been examined with so many samples before and after nutrient injection. All of the data illustrate that in nutrient-rich conditions, indigenous microbes of different communities accompanied by anaerobic organisms play an important role in oil emulsion and MEOR.

1. Introduction

Due to increasing energy demand and crude oil resource depletion, the development of enhanced oil recovery (EOR) faced opportunities and challenges (Tang et al., 2012). Microbial enhanced oil recovery (MEOR) processes employ microorganisms and microbial metabolites to achieve several aims, including lower interfacial tension between water and oil, reduced oil viscosity and enhanced permeability in reservoirs (Magot et al., 2000). Compared to other EOR techniques, MEOR have several advantages: lower energy consumption, no thermal processes, low environmental impact, and no chemical processes (Nilsen et al., 1996; Roling et al., 2004; Gauthier et al., 1992; Youssef et al., 2009). However, the technical performance in many field trials has been inconsistent (Voordouw et al., 1996; Orphan et al., 2000; Watanabe et al., 2000). Monitoring and controlling the conditions for optimal performance during the MEOR process is difficult for microbiologists. First, the injected strains are hard to track. Many studies have been performed in oil reservoirs (Voordouw et al., 1996; Orphan et al., 2000); however, few reports have monitored the changes in strains injected for MEOR (Voordouw et al., 1996). Second, the relationship between the microbial operation and oil yield is vague. Therefore, two major problems, the relationship between microbial community structure changes and enhanced recovery, and that between the composition of the microbial community and stimulation strategies, drew the attention of scientists and became crucial for the whole process (Watanabe et al., 2000; Zhang et al. 2010).

The underground oil reservoir is a complex ecosystem comprised of an undetermined number of bacteria, archaea, and some fungal species that combine to break down hydrocarbons, and to produce biosurfactants, gases and methane and to fix nitrogen (Magot et al., 2000). In the past two decades, culture-dependent and culture-independent approaches have been used to examine the microbial communities in oil reservoirs (Tang et al., 2012;

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Orphan et al., 2000). The development of molecular biology techniques provided several methods to study the complicated microbial community structures. 16S rRNA gene libraries, terminal-restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), real-time quantitative PCR and amplified ribosomal DNA restriction analysis (ARDRA) are the main technologies used to study microbial community structure (Tang et al., 2012; Voordouw et al., 1996; Orphan et al., 2000; Watanabe et al., 2000; Fan et al., 2010; Osborn et al., 2000; Filion et al., 2004; Smith and Osborn, 2009; Heyndrickx et al., 1996; Vaneechoutte et al., 1992). The 16S rRNA gene library was mainly used for free culture mode analysis of main populations in the microbial community, including the microbial category and proportion. Unfortunately, chimera, heteroduplexes, mutations and preferential amplifications, all types of artifacts, contribute large negative effects to the PCR amplification process, and make the microbial community structure results appear higher than the reality (Voordouw et al., 1996). These disadvantages can be offset by using T-RFLP to study the dynamic changes in a microbial community; this method is commonly used to analyze the structure and dynamic state variety of microorganisms. The credibility, reproducibility and robustness of T-RFLP generate its advantages, such as high-flux, speed, accuracy and resolution. Further, this method can be used to understand species composition (Orphan et al., 2000; Osborn et al., 2000), similarity relations and distances between communities. However, T-RFLP certainly has drawbacks for microbial community analysis (Osborn et al., 2000), for example, the method cannot accommodate hybrid or direct sequences and analysis of the clones. If the TRF fragments in the database do not match closely enough, identification results are not accurate to the species or even genus level. T-RFLP combined with the 16S rRNA gene library made up a useful method to overcome these drawbacks (Orphan et al., 2000; Filion et al., 2004). The same problems existed in Q-PCR and DGGE technologies. Q-PCR has problems, too, such as the requirement for fluorescent dye and high demand for primers (Smith and Osborn, 2009). DGGE requires poisonous formamide for the gradient gel electrophoresis; the detected DNA fragments are limited in size to 100–500 bp and pre-experiments must be performed. Amplified ribosomal DNA restriction analysis (ARDRA), based on restriction endonuclease digestion of amplified microbial 16S ribosomal DNA, is used to study the structure and fluctuation of microbial communities in the oilfield (Heyndrickx et al., 1996; Vaneechoutte et al., 1992). ARDRA bands are simple and easy to analyze, but the available information is scarce and difficult to use to assess species abundance and evenness. For the study of complex ecosystems such as underground oil reservoirs, a combination of complementary techniques such as a 16S rRNA gene library, T-RFLP combined with DGGE, Q-PCR and ARDRA generates more reliable results (Voordouw et al., 1996; Orphan et al., 2000; Watanabe et al., 2000; Fan et al., 2010; Osborn et al., 2000; Filion et al., 2004; Smith and Osborn, 2009; Heyndrickx et al., 1996; Vaneechoutte et al., 1992).

Indigenous microorganism structures of oilfields were analyzed, revealing that a flock of diverse microorganisms existed in the crude oil (mixture of oil and water), production water and ambient environment of the production wells (Tang et al., 2012; Fan et al., 2010; Cheng et al., 2006). All these studies expanded our understanding of the complexity and diversity of microbial communities in the oilfield (Voordouw et al., 1996; Orphan et al., 2000; Watanabe et al., 2000; Fan et al., 2010; Heyndrickx et al., 1996; Vaneechoutte et al., 1992; Cheng et al., 2006). However, microbial communities differ between individual oil reservoirs and oil wells, before and after nutrients are injected to activate the indigenous microorganisms (Youssef et al., 2009). The relationships between nutrient injections and bacterial communities was not clear; whether the microbial community changes after indigenous microorganism activation at one or three months can represent an entire change process in the oil-producing well. This problem had to be solved in this research, thus this study focused on the microbial communities and their changing processes before and after nutrition injection in the S12-4 oil well at the Shengli oilfield, by leveraging the advantages of multiple ecological methods to analyze the interactions between microorganisms, microbial communities and enhanced oil recovery. Understanding the microbial community distribution and the change process is crucial for the "microbial flooding" oil recovery process and for the mechanism of the single-well huff-and-puff technique and can provide theoretical guidance for MEOR (Tang et al., 2012).

2. Materials and methods

2.1. Sample collection and preparation

To enrich the microorganisms for the tertiary oil recovery process, the nutrient medium was injected into the S12-4 well (Clark and Jenneman, 1992), in the S12 block of the Shengli oilfield, Shandong province of eastern China, which is a site of endogenous microbial oil recovery (Capuano et al., 1995). The medium included carbon sources (glucose, 300 kg; molasess, 200 kg), phosphorus sources (inorganic and organic phosphates, such as potassium phosphate, 100 kg; trisodium trimetaphosphate, 50 kg), nitrogen sources (ammonium-containing compounds such as ammonium chloride, 100 kg; nitrates such as potassium nitrate, 20 kg; organic nitrogen sources such as peptone, 100 kg; amino acids and peptides, 30 kg) and other trace elements (including Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+}, 10 kg). The carbon sources, phosphorus sources, nitrogen sources and trace elements were dissolved in 20 T water and then injected into the S12-4 well continuously in one day with the ratio of 50:15:25:3. Later, 130 T water was injected by high-pressure pump to promote the nutrient diffusion in the oil reservoir. The construction technology described above has been carried out twice and an interval of seven months.

Samples of the oil–water mixture collected from the wellhead and analyzed in this study were obtained immediately (S4-1, after the second construction technology described above), or 7 months (S4-2), 9 months (S4-3), 14 months (S4-4), 17 months (S4-5) or 20 months (S4-6) after the nutrient addition. The production water (mixture of oil and water) was collected by 15 l plastic sampling bottles, then transported to the laboratory and allowed to stand for 2 h at room temperature to separate the oil and water phases. After separating the production water, the water phase was filtered by 0.22 μm membrane filters. The cells trapped on the membrane filter were used to extract DNA for microbial community analysis (Tang et al., 2012).

2.2. Chemical analysis

The cations and anions in the production water from the S12-4 oil well were analyzed by an ion chromatograph (LC-20A, Shimadzu, Kyoto) with a Shim-pack IC-A3 column for cation analysis and Shim-pack IC-C3 column for anions. The saturates, aromatics, resins and asphaltene contents of the crude oil were analyzed according to the Chinese Standard SYT 5119-2008 by fractionating with liquid–solid chromatography. Crude oil viscosity was determined *in situ* with a DV III Ultra viscometer (Brookfield, USA) (Chaillan et al., 2004).